

# Rapid micro-evolution and loss of chromosomal diversity in *Drosophila* in response to climate warming

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## Summary

Concern regarding the ecological impact of rapid global warming has encouraged research on climate-induced changes in biological systems. Critical problems, still poorly understood, are the potential for rapid adaptive responses and their genetic costs to populations. The *O* chromosomal polymorphisms of *Drosophila subobscura* have been monitored at a southern Palearctic locality experiencing sustained climate warming since the mid-1970s. Observations suggest that the population is rapidly evolving in response to the new environmental conditions, and has lost a significant amount of chromosomal diversity (18.3% in 16 years). These findings are consistent with results from another population of *D. subobscura*, which is also undergoing climate warming, and are in accord with what would be expected from latitudinal and seasonal patterns of the various inversions. In addition, data on the *O* chromosomal polymorphisms from other localities throughout the range of this species suggest that other populations vary similarly.

**Keywords:** biodiversity; chromosomal polymorphism; climate change; *Drosophila subobscura*; micro-evolution

## Introduction

The Greenhouse-induced increase in world temperatures is thought to be exceedingly rapid compared with prehistoric changes of similar magnitude (Vitousek, 1992; Schneider, 1993). In the face of such global environmental change, evolution may provide the only route to many species' survival. Indeed, laboratory experiments have shown that some bacteria populations rapidly adapt to changes in temperature by selection on *de novo* mutations (Bennett *et al.*, 1990). However, it is still uncertain whether higher eukaryotes, with smaller population sizes and longer generation times, can evolve rapidly enough to track climate change, or if they suffer from greater genetic loads resulting from the populations lagging behind the changing conditions (Holt, 1990; Lynch and Lande, 1993; Travis and Futuyma, 1993). In this study, we present an account of the changes of the *O* chromosomal-inversion polymorphisms of *Drosophila subobscura* in an area (O Pedroso mountain, NW Spain) that has been affected by a rapid, sustained climate warming ( $\Delta = 0.081^\circ\text{C}$  per year) since the mid-1970s.

*Drosophila* chromosomal-inversion polymorphisms have been extensively studied for about six decades (Krimbas and Powell, 1992), and several features suggest that they may be particularly suitable to detect evolutionary responses to climate warming: (1) *Drosophila* species have relatively narrow physical limits for resource utilization, temperature being one of the most important factors constraining their distributions (Parsons, 1991); (2) chromosomal arrangements of inversions can strongly determine the physiology and behaviour at the individual level (Wright and Dobzhansky,

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1946; Dobzhansky, 1947); and (3) due to the crossing-over suppression effect of inversions in structural heterozygotes, gene arrangements differing by one or more inversions can be considered to be independently evolving units. In the specific case of *D. subobscura*, compelling reasons suggest that a response of its chromosomal-inversion polymorphisms to climate warming might be expected. There is little doubt that climate is a major factor in determining the geographical variation of gene arrangements of the five acrocentric chromosomes of this species. Specifically, the relative frequency of some chromosomal arrangements has been found to co-vary with latitudinal (Menozzi and Krimbas, 1992) and inter-annual (Rodríguez-Trelles, 1993; Orengo and Prevosti, 1996; Rodríguez-Trelles *et al.*, 1996) temperature variation. Moreover, the same latitudinal clines have evolved independently in the Palearctic (the original area of distribution of the species) and in the recently colonized North and South America (Krimbas and Loukas, 1980; Prevosti *et al.*, 1988; Krimbas, 1992; Menozzi and Krimbas, 1992). The *O* chromosome in particular has been linked to thermo-tolerance-related traits. It contains the *Hsp70* gene family, which controls the most active heat shock proteins in this species (Moltó *et al.*, 1992), and exhibits direct responses to selection on heat tolerance in the laboratory (Quintana and Prevosti, 1991). Also, genes responsible for cold tolerance have been associated with the homologous chromosome 3 in *D. melanogaster* (Tucic, 1979). In natural populations, *O* chromosomal polymorphism changes with body size, a trait that has been shown to be negatively correlated with the July isotherm in the Palearctic (Misra and Reeve, 1964; Prevosti, 1966). Furthermore, a recent study of the seasonal changes of the inversion polymorphism of the O Pedroso population revealed that the relative frequency of common *O* arrangements varies following seasonal cycles (Rodríguez-Trelles *et al.*, 1996). When compared with their latitudinal patterns, the seasonal variation of these arrangements is consistent with what would be expected from temperature.

## Materials and methods

The study site was located at an altitude of 260 m on the south slope of O Pedroso mountain, 3 km from Santiago de Compostela (42.52°N; 80.33°W). Samples were collected by net sweeping over conventional mashed banana baits. The methods for chromosomal isolation and staining are described in Rodríguez-Trelles *et al.* (1996). Dates of collection were chosen to coincide with four significant seasons when natural populations of *D. subobscura* are active at this latitude: spring, early summer, late summer and autumn. The sampling spanned 16 years, from 1976 to 1980 (Fontdevila *et al.*, 1983) and from 1988 to 1991 (Rodríguez-Trelles *et al.*, 1996), making it one of the most exhaustive temporal studies of inversion polymorphism carried out in a natural population of *Drosophila* (see also Anderson *et al.*, 1991) and, to our knowledge, the only one to record year-to-year variation within different seasons. Up to 15 different *O* inversions were found, and this study focused on the most common ones over the study period:  $O_{3+4+7}$ ,  $O_{3+4}$ ,  $O_{ST}$  and  $O_{3+4+8}$ , whose frequencies add up to about 92% (relative frequencies of  $0.478 \pm 0.020$ ,  $0.183 \pm 0.011$ ,  $0.153 \pm 0.012$  and  $0.101 \pm 0.006$ , respectively: mean  $\pm$  1 s.e.). The relative frequencies of these four arrangements were obtained by scaling their numbers to the total number of arrangements collected in each sample, which reduces multicollinearity effects among them.

Chromosomal diversity was computed as  $1 - \text{IFR}$ , where IFR is the index of free recombination (Carson, 1955). This coefficient has the advantage of taking into account not only the number of inversions and their frequency, but also their length. The index quantifies the amount of euchromatin that is heterozygous for gene arrangements and it is basically a measure of heterozygosity weighted by the length of the inversions. A drop in  $1 - \text{IFR}$  means that less euchromatin is being tied up in inversions, and so the population is becoming less heterogeneous for chromosomal arrangements. Index values were estimated for each sampling date as:

$$1 - \text{IFR} = \sum_{\substack{i,j=1 \\ i \neq j}}^k p_i \cdot p_j \cdot (1 - l_{ij})$$

where  $p_i$  is the relative frequency of the  $O$  arrangement  $i$ ,  $p_j$  is the relative frequency of the  $O$  arrangement  $j$ ,  $l_{ij}$  is the proportion of  $O$  euchromatin that freely recombines between arrangements  $i$  and  $j$ , and  $k$  is the number of different  $O$  arrangements in the sample. To compute the index, each heterozygote class is considered in turn (for example,  $O_{3+4+8}/O_{3+4}$  first, then  $O_{3+4+8}/O_{\text{ST}}$ , and so on), and the amount of  $O$  euchromatin that is not involved in inversion loops is obtained by measuring the relative length of the inverted region on a microphotographic map of the paired polytene  $O$  chromosomes. These lengths were obtained from published tables (Krimbas and Loukas, 1979), which are currently considered to be the reference tables. Because we did not record individual genotypes but only inversion frequencies, we assumed Hardy-Weinberg heterozygote proportions. Data analysis involved multiple regression and Pearson correlation; we note here that analyses performed using non-parametric Spearman's correlation (not included) rendered similar results. Relative frequency of  $O$  arrangements data were angularly transformed before analysis. Candidate climatic variables for the multiple regression analyses included minimum, medium and maximum temperatures, relative humidity, precipitation, evaporation and insolation. Values for these variables were the averages for the 30-day period before each collection date, and were obtained at Labacolla Airport Meteorological Station,  $\sim 7$  km from the study site.

## Results

### *Climatic shifts*

The climate had become increasingly warmer at O Pedroso at the time of the study (Fig. 1). The most significant rise was in the average minimum temperature in summer ( $r = 0.68$ ,  $P = 0.00003$ ). Similar, but weaker trends were also shown by autumn average mean and minimum temperatures ( $r = 0.38$ ,  $P = 0.032$ ;  $r = 0.40$ ,  $P = 0.026$ , respectively); the annual and spring average minimum, mean and maximum temperatures, and summer average mean and maximum temperatures, were shown to be significant negative quadratic functions of year ( $P \leq 0.002$  in all cases). Over the sampling period (1976–91 for autumn samples and 1977–91 for the rest), all these temperatures showed significantly increasing linear trends ( $P \leq 0.034$  in all cases), except average summer maximum, which was only marginally significant ( $P = 0.087$ ). Similar results emerged when sampling years only were considered. Analyses were also performed using relative humidity, precipitation, evaporation and insolation. Relative humidity showed opposite trends to temperature, its annual average decreasing significantly over 1961–91 ( $r = -0.41$ ,  $P = 0.0239$ ) and 1976–91 ( $r = -0.53$ ,  $P = 0.037$ ), and its summer average over 1961–91 ( $r = -0.38$ ,  $P = 0.0328$ ). Annual precipitation also declined significantly over 1976–91 ( $r = -0.61$ ,  $P = 0.027$ ).

### *Chromosomal shifts*

The relative frequency of  $O_{\text{ST}}$  decreased significantly over the study period (Fig. 2),  $O_{3+4+8}$  exhibited a similar but weaker trend, and  $O_{3+4}$  increased significantly. The relative frequency of  $O_{3+4+7}$ , the most common arrangement, showed a non-significant shift throughout the years of the study. These overall temporal trends of the  $O$  arrangements conform with the within-season correlations between their relative frequencies and year (Table 1). Thus, correlation of  $O_{\text{ST}}$  with year was negative and significant within all four seasons. Similarly, correlations of  $O_{3+4}$  with year were positive, but only significant in spring.  $O_{3+4+8}$  showed negative correlation coefficients with year

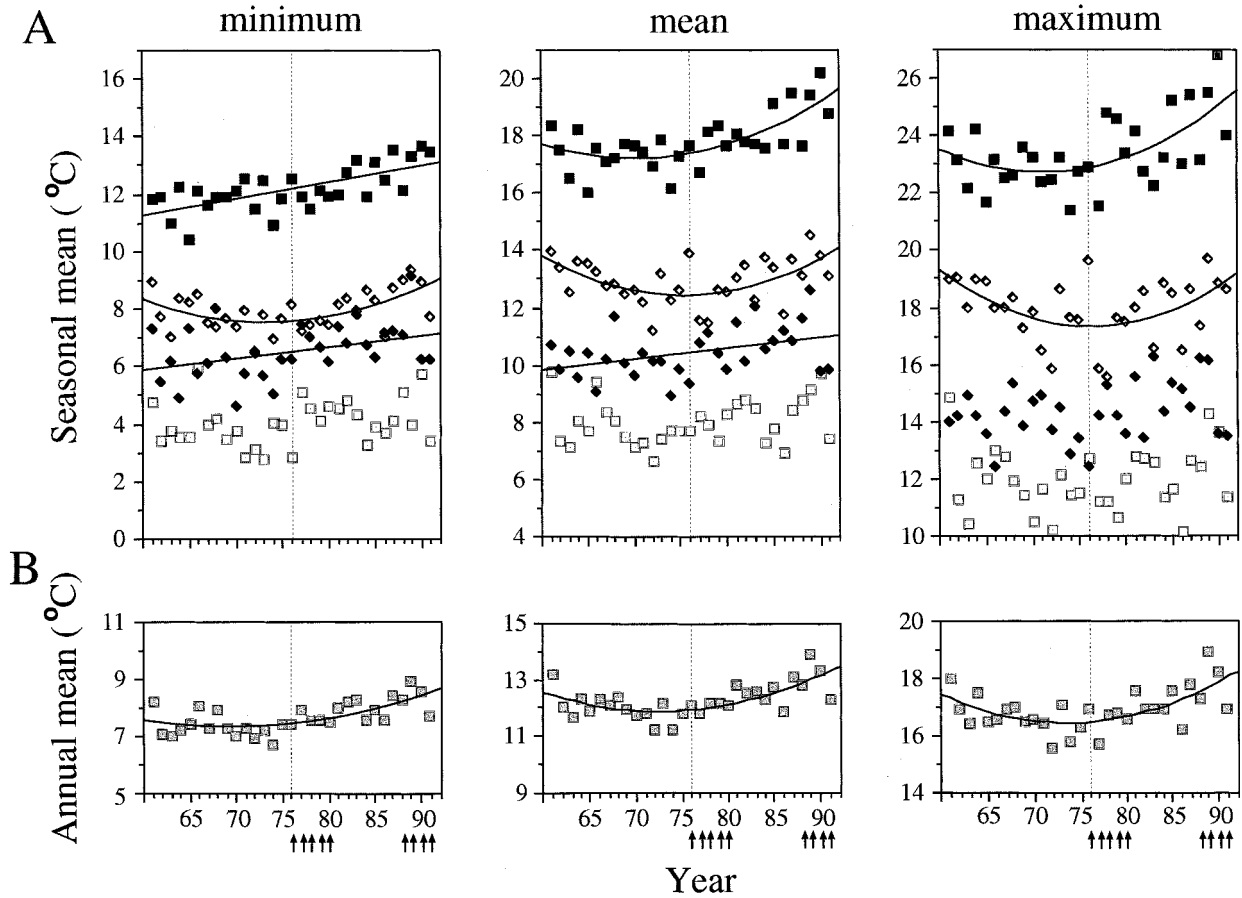


Figure 1. Variation in temperature in the study area over 31 years. (A) Seasonal and (B) annual average temperatures. Summer, spring, autumn and winter are indicated by solid squares, open diamonds, solid diamonds and open squares, respectively. Sampling period lies to the right of the dotted line. Arrows indicate sampling years. Analyses used polynomial regression with forward term addition. Only significant curves are shown.

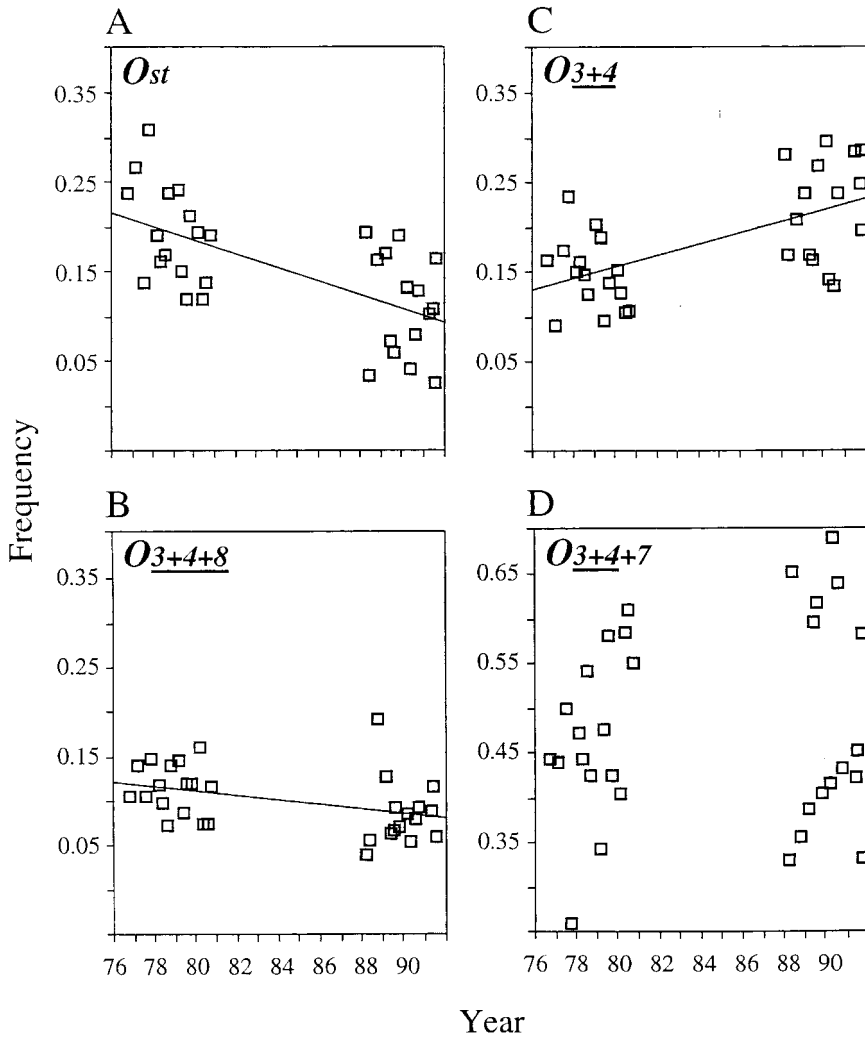


Figure 2. Temporal variation in the relative frequencies of the four most common *O* chromosomal arrangements in a natural population of *Drosophila subobscura* in NW Spain. Relative frequency of (A)  $O_{ST}$  and (B)  $O_{3+4+8}$  decreased with year ( $y = 15.30 - 0.008x$ ,  $R^2 = 0.41$ ,  $P = 0.0001$ ;  $y = 5.102 - 0.003x$ ,  $R^2 = 0.17$ ,  $P = 0.015$ , respectively), whereas (C)  $O_{3+4}$  increased significantly ( $y = -12.31 + 0.006x$ ,  $R^2 = 0.36$ ,  $P = 0.0004$ ) and (D)  $O_{3+4+7}$  did not show significant variation ( $P > 0.48$ ).

within all seasons, but none was significant, whereas  $O_{3+4+7}$  did not show any consistent nor significant relationship with year.

To investigate the influence of weather on the year-to-year variation of the *O* arrangements within each season, we carried out multiple regression analyses. For each season, we built minimal climatic models for  $O_{ST}$  and  $O_{3+4}$  (i.e. for the arrangements showing significant temporal variation). Minimal climatic models were obtained from initial models containing all the explanatory variables by stepwise elimination or addition of weather variables, and by retaining significant

Table 1. Within-season correlations between relative frequencies of *O* chromosome arrangements and year

Season	$O_{ST}$	$O_{3+4}$	$O_{3+4+8}$	$O_{3+4+7}$
Early summer ( $n = 7$ )	-0.781*	0.357	-0.599	0.492
Late summer ( $n = 7$ )	-0.921**	0.411	-0.596	0.711
Autumn ( $n = 9$ )	-0.818**	0.708*‡	-0.240	-0.141
Spring ( $n = 8$ )	-0.825*	0.910**	-0.638	-0.289

\* $P < 0.05$ ; \*\* $P < 0.01$ . ‡ Relationship significantly ( $P < 0.05$ ) better described by a negative quadratic function of year.

Table 2. Regressions of chromosomal arrangements exhibiting significant within-season temporal trends ( $O_{ST}$  and  $O_{3+4}$ ; see Table 1) on weather variables<sup>a</sup>

Season	<i>O</i> arrangement	Minimal climate model	% variance accounted for	$r_{\text{simple or partial}}$ for regression terms
Early summer ( $n = 7$ )	$O_{ST}$	$T_{\min}$	73.0*	-0.855*
Late summer ( $n = 7$ )	$O_{ST}$	$T_{\min}$	67.7*	-0.823*
Spring ( $n = 8$ )	$O_{3+4}$	$T_{\max}$ + <i>Hum</i>	90.1**	0.934** 0.884**

\* $P < 0.05$ ; \*\* $P < 0.01$ . <sup>a</sup> Minimal climatic models were obtained separately for each season (see text), and include factors with a significant partial correlation with the arrangement being analysed. Non-significant models are not included.

terms only (i.e. those exhibiting significant partial correlation with the inversion under study). To circumvent multicollinearity among explanatory variables, we did not include more than one temperature variable in the same regression model, choosing only the one showing the highest simple correlation with the inversion being analysed. Significant minimal climatic models were obtained for  $O_{ST}$  in early and late summer and for  $O_{3+4}$  in spring (Table 2). These models indicate that most of the inter-annual variation of  $O_{ST}$  in both summer periods can be accounted for by average minimum temperature. Given that the early and late summer models were not statistically fully independent, an influence of the levels of  $O_{ST}$  in early summer over those in late summer cannot be discarded. On the other hand, the frequency of  $O_{3+4}$  in spring was mainly associated with average maximum temperature and relative humidity (Table 2). The strong covariation between weather variables and time, which leads to problems of multicollinearity, and the reduced number of data points available within seasons, which ranged from 7 to 9, precluded further analysis to discriminate between the effects of weather and time on the frequency of the *O* arrangements.

No significant climatic models were found to account for the temporal trends of  $O_{ST}$  in spring and autumn (see Table 1). Although such trends could be independent from weather, it cannot be excluded that they resulted from time-delayed effects of climate in summer. Indeed,  $O_{ST}$  average summer and autumn frequencies were significantly autocorrelated ( $r = 0.844$ ,  $P = 0.017$ ). Similarly, the spring relative frequencies of  $O_{ST}$  were autocorrelated with those in late summer and autumn ( $r = 0.77$ ,  $P = 0.041$ ;  $r = 0.75$ ,  $P = 0.031$ , respectively).

A significant loss of *O* chromosomal diversity (18.3%) occurred over the study period (Fig. 3A). Depletion of chromosomal variation appeared to be strongly associated with climatic factors, especially with increasing minimum temperature (Fig. 3B). Within seasons, similar decreasing temporal trends in *O* chromosomal diversity were found in spring, early and late summer ( $r = -0.76$ ,  $P = 0.028$ ;  $r = -0.73$ ,  $P = 0.059$ ;  $r = -0.79$ ,  $P = 0.034$ , respectively), whereas corre-

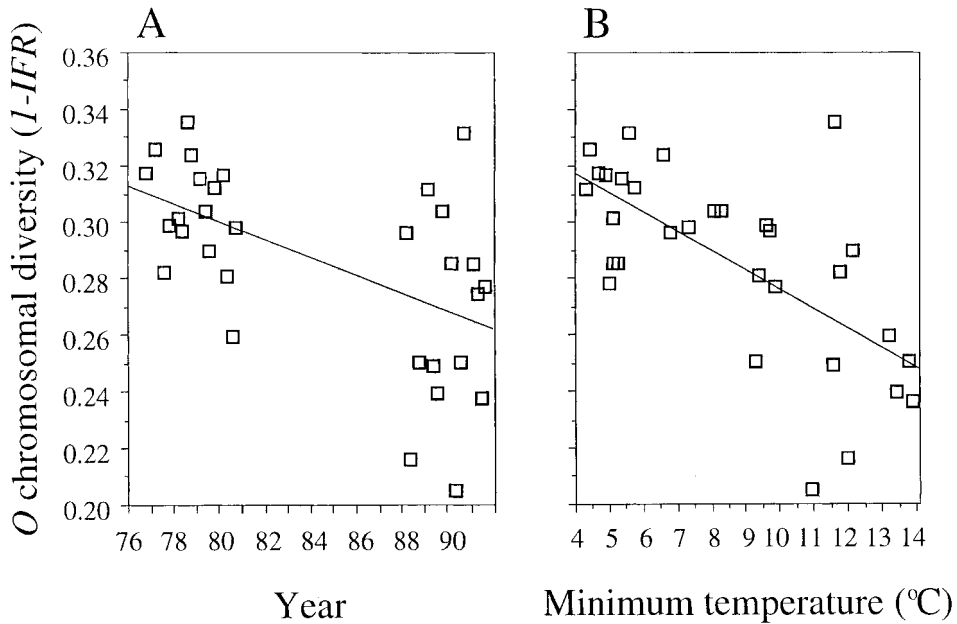


Figure 3. Relationships of *O* chromosomal diversity with (A) year and (B) average minimum temperature. Chromosomal diversity was estimated as  $1-IFR$ , where  $IFR$  is the index of free recombination (see text). The regression models were  $y = 6.59 - 0.003x$  for year ( $R^2 = 0.30$ ,  $P = 0.010$ ) and  $y = 0.35 - 0.007x$  for average minimum temperature ( $R^2 = 0.44$ ,  $P = 0.001$ ).

lations with climate variables were also significant for minimum temperature in early ( $r = -0.85$ ,  $P = 0.0166$ ) and, in particular, late ( $r = -0.90$ ,  $P = 0.0202$ ) summer.

## Discussion

Our results suggest that the *O* chromosomal-inversion polymorphism of *D. subobscura* is rapidly changing in response to climate warming at O Pedroso.  $O_{ST}$  is the arrangement showing the strongest shift, with a decline of 40.7% in its original frequency in 16 years. Consistent with this hypothesis, the inter-annual decline of  $O_{ST}$  is particularly pronounced at the end of the summer, the season when temperature increase over time has been most dramatic (see Fig. 1). This is not surprising, since among the *O* arrangements,  $O_{ST}$  declines the most in summer (Rodríguez-Trelles *et al.*, 1996), and exhibits the strongest correlation with latitude, prevailing in the colder boundaries of the distribution range of the species (Krimbas, 1992; Menozzi and Krimbas, 1992). Furthermore, flight activity levels of individuals carrying  $O_{ST}$  are the lowest at midday (Gosteli, 1991). In contrast,  $O_{3+4}$  has increased with time (Fig. 2C). This is consistent with the fact that this arrangement is the prevalent one in warmer regions (Krimbas, 1992; Menozzi and Krimbas, 1992), and that it currently achieves the second highest summer frequencies at O Pedroso (Rodríguez-Trelles *et al.*, 1996). The other two common arrangements,  $O_{3+4+8}$  and  $O_{3+4+7}$ , show weaker trends. In the case of  $O_{3+4+7}$ , this is somewhat unexpected, bearing in mind that this arrangement displays pronounced seasonal variation in the O Pedroso population (Rodríguez-Trelles *et al.*, 1996). However, since both arrangements also exhibit little latitudinal variation (Menozzi and Krimbas, 1992), factors other than temperature might be relevant.

Records on the temporal variation of *O* chromosomal polymorphism at about the same latitude as O Pedroso exist for three distant southern Palearctic populations (Gosteli, 1990; Zivanovic *et al.*, 1995; Orengo and Prevosti, 1996), all showing shifts of  $O_{ST}$  that parallel those described here. Weather records included in Orengo and Prevosti's (1996) study, which was limited to seven autumn collections in Barcelona, allowed us to further examine the variation of  $O_{ST}$  in this north-eastern area of Spain. When considering the data obtained since the 1970s,  $O_{ST}$  decreased significantly ( $r = -0.96$ ,  $P = 0.002$ ,  $n = 6$ ), spring-fall average temperature increased significantly ( $r = 0.95$ ,  $P = 0.004$ ,  $n = 6$ ), and the two variables were significantly correlated with each other ( $r = -0.87$ ,  $P = 0.02$ ,  $n = 6$ ), mimicking the patterns we observed. The parallel decline in  $O_{ST}$  in these distant populations suggests that the arrangement is retreating from the southern Palearctic. Interestingly,  $O_{ST}$  appears to be showing the opposite trend in the high northern Palearctic, specifically because *D. subobscura* has pushed its margin of distribution by some 500 km to the north in Scandinavia (from latitude 61.0°N to 64.0°N) since about 1970 (Saura, 1994). Similar to what has been hypothesized to have occurred after the last glaciation (Krimbas, 1992; Menozzi and Krimbas, 1992), this expansion is presumed to be mediated by  $O_{ST}$ , the prevailing *O* arrangement in this region. Evidence from the fossil record suggests that previous shifts in many species' boundaries were linked to major climate changes. In light of our findings, the shift in the distribution of  $O_{ST}$  in the Palearctic could similarly be associated with the speed of climate warming in the Northern Hemisphere since the mid-1970s (Jones *et al.*, 1986; Wallace *et al.*, 1995; Johannessen *et al.*, 1996).

Our results show that rapid changes in the *O* inversion polymorphism at O Pedroso occur with a significant loss of chromosomal diversity. In addition to this loss, seasonal episodes of rapidly shifting selection by temperature over at least 16 years (> 80 generations) are expected to erode genetic variation within arrangements. Theoretical research suggests that under directional or cyclical selection, reduced additive genetic variance causes the mean phenotype to lag behind the changing optimum, increasing the evolutionary load (Lande and Shannon, 1996). The case of  $O_{ST}$  is paradigmatic. The outstanding parallelism between latitudinal, daily, seasonal and long-term variation of  $O_{ST}$  strongly suggests that, within this arrangement, it evolved a genetic architecture that performs optimally in cold environments. Since the study population lies in the warmer territories of the distribution range of  $O_{ST}$  (Krimbas, 1992; Menozzi and Krimbas, 1992), this arrangement may be particularly sensitive to climate warming, suffering from greater genetic loads. This agrees with findings at O Pedroso which show that this arrangement exhibits reduced levels of nucleotide diversity (Rozas *et al.*, 1995), and that the gametic disequilibrium between the loci Leucine aminopeptidase and Peptidase-1 inside  $O_{ST}$  changes following seasonal cycles in this population (Rodríguez-Trelles, 1993). This interpretation is consistent with theoretical results obtained by Bulmer (1980) showing that non-random association between genes usually builds up during the course of long-term directional selection. Since crossing-over between inversions is almost completely restricted to structural homozygotes, the chances of  $O_{ST}$  releasing new combinations of alleles fast enough to closely track the rapid climate warming diminish with its own frequency. Ultimately, depleted chromosomal variation may reduce the likelihood of the population to cope with environmental stochasticity, jeopardizing its very existence.

Abrupt Prehistoric climatic excursions have been linked to milestones in evolution (Kerr, 1992). However, the predicted global temperature increase of up to 5°C within the next 100 years is unprecedentedly rapid. In such a scenario, biological systems that are currently at equilibrium with their environment will have to deal with ecological circumstances that no longer fit those in which they evolved, with potential catastrophic consequences. These may be particularly critical for ectotherms like insects, which have no physiological or biochemical mechanisms to prevent their body temperature from tracking the environmental temperature. If the actual levels of climate



warming are enough to derail the evolutionary walk of a generalist among *Drosophila*, such as *D. subobscura*, what is actually occurring to many other specialist and non-colonialist insects? Clearly, more research on this subject is urgently needed to clarify current and potential evolutionary effects of climate change, and their costs for ecosystems.

### Acknowledgements

We are indebted to Ray Huey, Brad A. Hawkins, Mauro Santos, Dick R. Hudson, Steven A. Frank, Francisco J. Ayala, Larry D. Mueller, Robin Bush, Rosa M. Tarrío, Rosa M. Vidanes and Jordi Bascompte for helpful comments on the manuscript, and Júlio M. Maside for assistance in field sampling. F.R.-T. was supported by NIH GM grant 42397 to F.J. Ayala, and M.A.R. by the Spanish Ministry for Education and Culture (postdoctoral fellowship FP94 51641052 and CICYT grant AMB96-1161 to A. Gómez-Sal).

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